

Safety Evaluation of Silkworms Fed Leaves Sprayed with Commonly Used Pesticides in Fruit Mulberry Production

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Abstract: This study was to evaluate the safety of commonly used pesticides on mulberry for commonly used silkworms in Chongqing, and to provide reference for pest control and scientific pesticides using in mulberry orchards in silkworm areas. 5 fungicides and 5 insecticides were sprayed on mulberry, mulberry leaves were used to feed the 3rd instar silkworms 15-40 days later. The results showed that there was no symptoms of poisoning and death to the 3rd instar silkworms and there was no difference in economic indexes between treatment groups and control group, the treatment groups were separately sprayed with 50% carbendazim WP, 70% thiophanate-methyl WP, 10% difenoconazole WDG, 50% procymidone WP, 50% boscalid WDG, 10% imidacloprid WP and 8% chlorfenapyr ME, this showed that these pesticides were safe for sericulture, to avoid affecting the quality of cocoons, leaves should be picked 15 days later. However, there had potential safety risks for silkworms when spraying with 80% cyromazine WDG and 25% thiamethoxam WDG, in order to ensure the safety of silkworms, spraying must be done more than 40 days. 2.5% bifenthrin EW had highly toxic and a long residual period to silkworms, it has been forbidden to use in mulberry fields for sericultural production.

1 INTRODUCTION

Bombyx mori belongs to Lepidoptera and is very sensitive to most chemical pesticides. Every year, silkworm pesticide poisoning occurs in the sericulture area, which is caused by improper using of pesticides, causing great economic losses to the silkworm farmers (Li, Zhang, Zhong. 1998), (Ma, Wang, Wang, 2005), (Yu, Wang, Wu, 2011), (Wang, Zhang, She. 2021). At present, chemical control is still one of the main methods of pest management in mulberry fields (Wang, Zhang, She. 2021), (Song, Chen, Luo. 2020). When using pesticides to control pests and diseases in mulberry orchards, the selection and spraying methods of pesticides were not appropriate, pesticides will pollute mulberry orchards and the rearing environment of silkworms by direct spraying, which will cause pesticide poisoning for silkworms, and have a great impact on economic benefits of silkworm farmers. For example, 10% difenoconazole WDG is often used to control

mulberry fruit sclerotiniosis in mulberry production. However, Mingxiao Lv (Lv, Zhang. 2014) found that 400 g/L difenoconazole SC is highly toxic to silkworms by using the leaf leaching method, and believes that 400 g/L difenoconazole SC is not suitable to be used in mulberry fields. Therefore, when using pesticides to control diseases and pests, the impacts of various pesticides on the growth and development of silkworms should be fully considered to ensure the sustainable development of mulberries and silkworms. To understand the effects of commonly used pesticides on silkworm growth and improve the production safety awareness of cocoon peasant households, this experiment was carried out in 2020. Pesticides were sprayed at commonly used concentrations in production on mulberry, mulberry leaves were used to feed the 3rd instar silkworms. The symptoms of poisoning, the instar development, body weight, cocooning rate, cocoon weight, cocoon shell rate, rate of dead cocoons were systematically investigated, then comprehensive effects of 10 pesticides on

sericulture production were known. This is particularly significant to improve the awareness of safety production for cocoon peasant households, to use pesticides scientifically, and to ensure the sustainable development of the sericulture industry.

2 MATERIALS AND METHODS

2.1 Experiment Materials

The experiment was carried out at the Ganning Base of the Chongqing Three Gorges Academy of Agricultural Sciences. The experimental site is 325 m above sea level. Flat terrain, uniform fertility, and

consistent field management were chosen for experiments. The test soil is sandy loam soil, with thickness equal to or higher than 1.0 m.

The mulberry variety used in this test was Hu mulberry with planting density of 9 000 plants /hm², which is managed according to conventional water and fertilizer management.

The silkworm species tested in this experiment were Lianguang No.2, the original species were supplied by Chongqing Sericulture Science and Technology Research Institute.

The pesticides tested in this experiment were 10 commonly used pesticides in mulberry orchards, and the information was shown in table 1.

Table 1: Pesticides information

Pesticides	Content/Formulation	Dilution times	Manufacturer
Darbendazim	50%WP	800	Sunong (Guangde) Biotechnology Co., Ltd.
Thiophanate-Methyl	70%WP	1000	Zhenjiang Jiansu Pesticide Chemical Co., Ltd.
Difenoconazole	10%WDG	1000	Yifan Biological Technology Group Co., Ltd.
Procymidone	50%WP	1000	Jiangxi Heyi Chemical Co., Ltd.
Boscalid	50%WDG	1500	Qingdao Otis Biotechnology Co., Ltd.
Imidacloprid	10%WP	2000	Shandong Jiacheng Crop Science Co., Ltd.
Chlorfenapyr	8%ME	2000	Guangxi Tianyuan Biochemical Co., Ltd.
Cyromazine	80%WDG	2000	Guangdong Zhongxun Agricultural Science Co. Ltd
Bifenthrin	2.5%EW	2000	Chengdu Kelilong Biochemical Co. Ltd.
Thiamethoxam	25%WDG	2000	Jiangsu Changqing Agrochemical Co. Ltd.

2.2 Field Application Experiment

In 2020, pesticides were selected for testing listed in table 1. The test was carried out to evaluate the safety of the most frequently used pesticides on mulberry for silkworms of commonly used in Chongqing. Control was sprayed with clean water. The shape of the plot was square with basically the same area, around which guardrows were established. Pesticides were sprayed thoroughly and evenly on the mulberry trees by using electric sprayers after the dew dried up in the morning.

2.3 Safety Evaluation of Pesticides on Mulberry

At 1, 3, 5, 7, 10 and 15 days after application, the phytotoxicity on mulberry trees was observed. The main manifestations of phytotoxicity were

discoloration, necrosis, growth delay, wilting and deformity etc. According to these, safety of pesticides on mulberry was evaluated (Yang, Xie, Liao, 2021).

2.4 Safety Evaluation of Pesticides for Silkworms

In the autumn of 2020, the safety of the most frequently used pesticides on mulberry for commonly used silkworms in Chongqing was evaluated. Five insecticides and five fungicides which were commonly used in fruit mulberry production were sprayed on mulberry trees, control was sprayed by clean water. Mulberry leaves were picked to feed the same 3rd instar silkworms at 15 days, 20 days, 30 days and 40 days later. Each treatment had 3 replicates, and each replicate was fed with 50 silkworms. Observed the physiological reaction of silkworms, the number of dead

silkworms was investigated 24 hours later. The silkworms were touched with a small brush for different experimental treatments, if they did not move or could not react normally, they were regarded as dead, the surviving silkworms continued to be fed until cocooning. The instar development, body weight, cocooning rate, cocoon weight, cocoon shell rate, rate of dead cocoons were investigated.

2.5 Data Analysis

Statistical analysis of data was performed by using IBM SPSS 16.0. One-Way ANOVA was used to conduct difference analysis, and the data was expressed $\bar{x} \pm s$ (n=3).

3 RESULTS AND ANALYSIS

3.1 The Safety Evaluation Result of the Medicament on Mulberry

At 1, 3, 5, 7, 10 and 15 days after application, the phytotoxicity on mulberry trees was investigated. The results showed that there was no symptom of discoloration, necrosis, growth retardation, wilting

and malformation in the mulberry treated with different chemicals, and there was no significant difference between treatment groups and control group in the growth of mulberry trees.

3.2 Evaluation Results of Acute Poisoning of Mulberry Leaf to Silkworms

Acute poisoning of silkworms was shown in table 2, the results showed that there was no symptoms of poisoning and death to the 3rd instar silkworms, which were fed with mulberry leaves those were harvested after spraying with 50% carbendazim WP, 70% thiophanatemethyl WP, 10% difenoconazole WDG, 50% procymidone WP, 50% boscalid WDG, 8% chlorfenapyr ME, 10% imidacloprid WP, 80% cyromazine WDG at an interval of 15 to 40 days. All the silkworms died when they were sprayed with 2.5% bifenthrin EW for 24 hours. In these processing areas prayed with 25% thiamothoxam WDG for 5 to 20 days, all the silkworms died after feeding for 96 h, and spray interval of which was 30 to 40 days, death rates were 12% to 14% after feeding for 96 h.

Table 2: Questionnaire of acute poisoning of silkworms.

Pesticides	Interval times(d)	Medication time 24h		Medication time 48 h		Medication time 72h		Medication time 96 h	
		Living silkworm quantity (head)	Death rate (%)	Living silkworm quantity (head)	Death rate (%)	Living silkworm quantity (head)	Death rate (%)	Living silkworm quantity (head)	Death rate (%)
Carbendazim	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	40	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Thiophanate-Methyl	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	40	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Difenoconazole	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	40	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Procymidone	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	40	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Boscalid	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Imidacloprid	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a

	40	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Chlorfenapyr	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	40	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Cyromazine	15	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	20	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	30	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
	40	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a
Bifenthrin	15	0.00 d	100.00 d	-	-	-	-	-	-
	20	0.00 d	100.00 d	-	-	-	-	-	-
	30	0.00 d	100.00 d	-	-	-	-	-	-
	40	0.00 d	100.00 d	-	-	-	-	-	-
Thiamethoxam	15	10.00 c	80.00 c	8.67 d	82.67 d	4.67 e	90.67 e	0.00 d	100.00 d
	20	33.00 b	34.00 b	25.33 c	49.33 c	17.33 d	65.33 d	0.00 d	100.00 d
	30	50.00 a	0.00 a	50.00 a	0.00 a	47.00 c	6.00 c	43.00 c	14.00 c
	40	49.67 a	0.00 a	47.67 b	4.67 b	47.67 b	4.67 b	44.00 b	12.00 b
CK		50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a	50.00 a	0.00 a

Values followed by different letters at the same column indicate significant difference ($P < 0.05$), and those followed by the same letters indicate no significant difference ($P \geq 0.05$), the same as below.

3.3 Effects on the Development of Silkworms

Mulberry leaves were picked to feed the 3rd instar silkworms 15, 20, 30, and 40 days later after application. The results showed that there was no significant difference between treatment groups and control group in the development of silkworms except for those the mulberry leaves sprayed with 2.5% biphenthrin EW and 25% thiamethoxam WDP, and there was no acute poisoning death.

3.4 Effects on Economic Indicators of Silkworms

The effects on economic indicators of silkworms were shown in table 3, the results showed that economic indexes of treatment groups which spraying with 50% carbendazim WP, 70% thiophanate-methyl WP, 10% difenoconazole WDG,

50% procymidone WP, 50% boscalid WDG were similar to those of the control group. Economic indexes of treatment groups which spraying with 10% imidacloprid WP and 8% chlorfenapyr ME were similar to those of the control group, but the cocooning rate of 10% imidacloprid WP and 8% chlorfenapyr ME groups at an interval of 15 days were slightly lower than the control, cocooning rate of those groups whose interval time was more than 20 days was similar to that of the control group. Economic indicators of 80% cyromazine WDG group whose leaves were picked 15 to 30 days later were significantly lower than that of the control group, reared silkworms at 40 days later, the performance of sericulture was similar to control group. Economic indexes of treatment groups which spraying with 25% thiamethoxam WDG for 30~40 days was significantly lower than that of the control group.

Table 3: Effects on economic indicators of silkworms.

Treatments	The cocooning period				Economic indicators			
	Interval times(d)	Cocoon Number (heads)	Cocooning rate (%)	Cocoon weight(g)	Cocoon shell weight (g)	Cocoon shell rate (%)	Pupa weight(g)	Death pupa Rate (%)
Carbendazim	15	146.67 ab	99.32 a	1.69 defg	0.35 bc	20.62 bcd	1.34 def	3.41 d
	20	133.00 j	99.25 a	1.66 de	0.34 bc	20.67 bcd	1.32 de	3.77 d
	30	145.33 ab	99.54 a	1.65 d	0.34 bc	20.33 bcd	1.31 d	4.59 cd
	40	147.00 a	99.54 a	1.66 de	0.33 bc	20.29 bcd	1.32 de	4.99 cd
Thiophanate-Methyl	15	145.00 abc	99.09 a	1.67 def	0.34 bc	20.47 bcd	1.33 de	3.69 d
	20	134.00 ij	99.26 a	1.69 defg	0.34 bc	20.41 bcd	1.34 def	3.48 d
	30	140.67 defg	99.30 a	1.69 defg	0.35 bc	20.81 d	1.34 def	3.55 d
	40	140.67 defg	99.53 a	1.67 def	0.34 bc	20.62 bcd	1.33 de	4.97 cd

Difenoconazole	15	139.00 defg	99.29 a	1.69 defg	0.35 bc	20.65 bcd	1.34 def	3.60 d
	20	138.33 defg	99.29 a	1.70 defg	0.35 bc	20.39 bcd	1.35 def	4.58 cd
	30	146.00 ab	99.10 a	1.67 def	0.34 bc	20.40 bcd	1.33 de	3.89 d
Procymidone	40	146.33 ab	99.32 a	1.68 def	0.34 bc	20.38 bcd	1.33 de	5.24 cd
	15	136.00 hij	98.09 ab	1.68 defg	0.34 bc	20.64 bcd	1.34 de	3.47 d
	20	141.67cde	99.07 a	1.68 def	0.34 bc	20.42 bcd	1.33 de	4.23 d
Boscalid	30	147.00 a	99.10 a	1.71 efg	0.36 bc	20.80 d	1.36 def	4.08 d
	40	141.33 def	99.30 a	1.68 defg	0.34 bc	20.33 bcd	1.34 def	4.48 cd
	15	140.67 defg	99.06 a	1.67 def	0.34 bc	20.69 bcd	1.33 de	3.81 d
Imidacloprid	20	145.33 ab	98.87 a	1.68 defg	0.34 bc	20.69 bcd	1.33 de	4.38 d
	30	141.00 defg	99.29 a	1.73 g	0.35 bc	20.35 bcd	1.38 f	4.47 cd
	40	145.67 ab	99.54 a	1.68 defg	0.34 bc	20.55 bcd	1.34 de	4.58 cd
Chlorfenapyr	15	136.33 hij	96.72 bc	1.67 def	0.34 bc	20.21 bcd	1.34 def	3.97 d
	20	135.33 hij	99.03 a	1.70 defg	0.35 bc	20.36 bcd	1.35 def	3.92 d
	30	144.33 abcd	99.09 a	1.72 fg	0.35 bc	20.52 bcd	1.37 ef	5.07 cd
Cyromazine	40	146.00 ab	99.55 a	1.66 de	0.34 bc	20.41 bcd	1.32 de	4.79 cd
	15	137.67 fgh	97.18 bc	1.66 de	0.34 bc	20.55 bcd	1.32 de	4.37 d
	20	138.67 efg	98.82 a	1.70 defg	0.35 bc	20.58 bcd	1.35 def	3.82 d
Thiamethoxam	30	143.00 bcd	99.54 a	1.68 defg	0.34 bc	20.73 cd	1.33 de	5.13 cd
	40	147.00 a	99.55 a	1.70 defg	0.34 bc	20.24 bcd	1.35 def	4.77 cd
	15	63.33 l	76.39 e	1.38 a	0.28 a	20.47 bcd	1.10 a	16.28 a
CK	20	65.33 l	85.65 d	1.45 b	0.28 a	19.67 ab	1.16 b	12.78 b
	30	124.67 k	95.92 c	1.46 bc	0.28 a	19.76 abc	1.18 bc	7.76 c
	40	143.33 abcd	98.17 ab	1.69 defg	0.34 bc	20.61 bcd	1.34 def	5.81 cd
CK	30	136.00 hij	98.07 ab	1.50 c	0.28 a	19.05 a	1.21 c	5.17 cd
	40	137.33 ghi	98.80 a	1.68 defg	0.34 bc	20.49 bcd	1.34 def	4.62 cd
CK		142.67 ab	99.32 a	1.67 defg	0.35 bc	20.52 bcd	1.34 def	3.42 d

4 CONCLUSION AND DISCUSSION

The last treatment for mulberry fruit sclerotiniosis and gall midge of mulberry is 15~20 days before mulberry ripening, which is more than 30 days for rearing silkworm in spring. From security considerations of silkworms, commonly used fungicides for disease prevention in production are 50% carbendazim WP, 70% thiophanatemethyl WP, 10% difenoconazole WDG, 50% procymidone WP, 50% boscalid WDG. The leaves were picked 15 to 30 days after spraying these fungicides, and fed to the 3rd instar silkworms. There was no symptom of poisoning and death, and rearing performances were similar to those of the control, which indicated these fungicides are safe for sericulture. However, in the paper of Mingxiao Lv^[6], the acute toxicity of 400 g/L difenoconazole SC on silkworm is classified as "high toxicity", so the safety interval should be considered when using difenoconazole in production. In this study, it was found that the spraying with 10% difenoconazole WDG 15 days is safe and non-toxic to silkworms.

Five insecticides and five fungicides which were the most frequently used in fruit mulberry

production were sprayed on mulberry to evaluate the safety of silkworms. The results showed that there was no symptoms of poisoning and death to the 3rd instar silkworms which were fed with mulberry leaves which were sprayed with the five fungicides (50% carbendazim WP, 70% thiophanate-methyl WP, 10% difenoconazole WDG, 50% procymidone WP, 50% boscalid WDG) and two insecticides (10% imidacloprid WP and 8% chlorfenapyr ME) 15 to 30 days, except that the cocooning rate of the insecticide treatment groups were slightly lower than the control, mulberry leaves of which picked on 15 d after pesticide application were used to feed the 3rd instar silkworms, there was no difference in the cocooning period results and economic indexes between the treatment groups and the control group. However, there had potential safety risks for silkworms when spraying with 80% cyromazine WDG and 25% thiamethoxam WDG on mulberry, to ensure the safety of silkworms rearing, the interval period of spraying must be more than 40 days. 2.5% bifenthrin EW has highly toxic and has a long residual period to silkworms, all silkworms died when mulberry leaves were fed for 24 hours with spraying 40 days interval. These results indicated that mulberry leaves which were sprayed

with the five commonly used fungicides, 10% imidacloprid WP and 8% chlorfenapyr ME in mulberry orchards are safe for sericulture, but 10% imidacloprid WP and 8% chlorfenapyr ME can be used in sericultural production, the leaves should be picked at intervals of 15 days to avoid affecting the quality of cocoons, and the mulberry leaves sprayed with 80% cyromazine WDG, 25% thiamethoxam WDG and 2.5% bifenthrin EW had potential safety hazards for sericulture, so they have been forbidden to use in mulberry fields for sericultural production.

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